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ABSTRACT

Soils around the world are being exposed to weather events which are unprecedented in recent history. To maintain the delivery of soil-related ecosystem services and to promote greater soil resilience it is essential to understand how plant-soil systems respond to these extreme events. In this study we replicated a recent period of extreme rainfall and prolonged spring flooding in a temperate grassland which had no previous history of flooding. Intact soil mescosms (Eutric Cambisol) 1 kg weight were subjected to a simulated long-term spring flood (15°C, 2 months) and maintained in the light with above ground indigenous vegetation (*Lolium perenne* L.) or dark with and without indigenous vegetation to simulate different flood typologies. In comparison to a no-flood control treatment, nutrient cycling, water quality, air quality (greenhouse gas emissions), habitat provision and biological population regulation shifts were evaluated. Flooding induced a rapid release of nutrients into the soil solution and overlying floodwater, resulting in potential nutrient losses up to 15 mg Fe, 16 mg NH_4^+, 360 mg DOC and 28 mg DON, per mesocosm. The presence of plants increased the

rate of nutrient release (especially P), with the effects magnified when light transmission through the floodwater was restricted (1.3 mg P vs 0.2 mg P, per mesocosm). Flooding induced a rapid decline in redox potential and subsequent production of CH_4, especially in the darkened treatments (10 and between 11–16 times higher than the control, without and with light restrictions, respectively). Upon removal of the floodwater, the accumulated NH_4^+ was nitrified leading to a shift in greenhouse gas emissions, from CH_4 to N_2O emissions. N_2O was only significantly produced in the mesocosms kept under light restrictions (13 times higher than in other two treatments). Flooding eliminated earthworms, reduced grass production after soil recovery (from 28 g for control mesocosms to 11 g and < 1 g for flooded mesocosms without and with light restrictions, respectively). Soil microbial biomass was also reduced (up to a 22–27 % of the total PLFAs) and flooding induced shifts in microbial community structure, particularly a loss of soil fungi. The soil fungi content quickly recovered (4 weeks) when light was not restricted during the flood period, however, no such recovery was seen in the darkened treatments. Overall, we conclude that extreme flood events cause rapid and profound changes in soil function. Both the impact of the flooding and the time to recover is exacerbated when light is restricted (e.g. in sediment laden floodwater). In addition, our results suggest that the presence of flood-resilient plants can mitigate against some of the negative impacts of flooding on soil functioning.

Keywords: Climate change, nitrate, PLFA profiling, tipping point, waterlogging tolerance, ^{33}P

48 **1. Introduction**

49 Against a backdrop of progressive climate change, there is increasing evidence that many
50 ecosystems are also experiencing more extremes in weather (e.g. heat, droughts, floods and
51 ground level ozone; Easterling et al., 2000). Within Western Europe, recent quasi-stochastic
52 extremes in air temperature and changes in the periodicity and intensity of rainfall have been
53 directly linked to climate change (Allan, 2011; Pall et al., 2011; Jones et al., 2012). To a large
54 extent this appears to be due to behavioural changes in the North Atlantic oscillation weather
55 system which is influenced by the polar vortex and Pacific jet stream (Murphy et al., 2009).
56 Alterations in this weather system have now been linked to numerous unprecedented storms
57 and long-term flooding events within the UK (Met Office, 2014). Examples of these include
58 those seen in the summer of 2012 and in the winter of 2013-2014 when large areas within the
59 England and Wales remained flooded for 10-12 weeks, in some cases under several metres of
60 floodwater (McEwen et al., 2014). Although some of these areas clearly lie within flood
61 plains which have a long history of inundation, importantly, other areas with no previous
62 documented history (>150 years) of flooding were also affected (Clout, 2014; Thorne, 2014).
63 These agroecosystems were severely impacted by the long-term flooding, typically resulting
64 in complete crop failure, loss of soil functions and in some cases a complete loss of topsoil
65 due to water erosion (Natural England, 2014). After the floodwater receded, it was also
66 apparent that there was a lack of appropriate interventions to reverse the negative impacts on
67 soil functioning as the impacts of extreme flooding were poorly understood. There is therefore
68 a clear need to understand the impact of extreme precipitation events on the soils with no
69 previous history of flooding.

70 Depending on the typology of the flood event, i.e. duration, depth, origin of the water
71 (Sánchez-Rodríguez et al., 2018), and the soil/vegetation combination, prolonged inundation
72 is expected to impact upon soil functions to different extents. Bünemann et al. (2018) has
73 recently reviewed the multiple aspects and definitions of soil quality. In this manuscript, we

define soil quality as “the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health” (Doran and Parkin, 1994, 1996). While short term flooding (<7 d) may have limited impact on soil functions, extreme flood events (> 2 months) may have major consequences on the delivery of a range of ecosystem services both during the flood itself and during the recovery phase (Niu et al., 2014), such as biomass production, biodiversity and water quality and supply. These soil-based ecosystem services are associated with soil functions, including habitat provision for roots and soil organisms, element cycling, decomposition, maintenance of soil structure, regulation of biological populations, water cycling and organic matter cycling (Bünemann et al., 2018).

After a few weeks of flooding, anaerobic conditions prevail in flooded grassland soils, facilitating the solubilisation of reduced elements (e.g. Fe, Mn; Schalenghe et al., 2007) and alterations in nutrient (P, Fe, N, C) cycles, including the release of soluble C and N (Jones et al., 2009) and promoting the loss of nutrients (via leaching or to the overlying water column). In addition, light ingress can be reduced preventing photosynthesis and inducing plant senescence (Mommer et al., 2005a; Shaw et al., 2013) depending upon the nature of the floodwater (particles suspended within the water column and deposited onto the foliage surface). Under these anaerobic conditions certain greenhouse gas (GHG) emissions could be stimulated; CH_4 production and N_2O emissions related to denitrification (transformation of NO_3^- to N_2O/N_2 mediated by soil bacteria) are anticipated (Hou et al., 2000), but N_2O production due to nitrification (transformation of NH_4^+ to NO_3^- mediated by soil bacteria) will be reduced as it is an aerobic process (Norton, 2008). These conditions facilitate the decomposition of organic matter derived from the senescing plant material and obligate-aerobic components of the microbial community. Soil microbial communities and some soil microorganisms, e.g. fungi, could be negatively affected if the inundation is prolonged, while other taxonomic groups, e.g. Gram+ bacteria, may prove more resistant (Ferré et al., 2012).

100 Phospholipid-derived fatty acids (PLFAs) as bioindicators of different taxonomic groups have
101 been traditionally used to assess these alterations in microbial communities of flooded soils
102 (Bossio and Scow, 1998). Finally, eutrophication of water bodies and a decline in soil
103 functionality and associated ecosystem services are expected after a prolonged flooding event
104 (Scalenghe et al., 2012; Brun and Barros, 2013; Shaw et al., 2013).

105 Until now, flooding experiments have largely focused on the options to mitigate flood
106 risk. They have usually been short term laboratory studies with disturbed soil, without
107 vegetation and non-extreme flooding, as reviewed by Brun and Baros (2013) and Shaw et al.
108 (2013). There is a lack of information about the magnitude of long-term flooding and its
109 effects on agricultural grasslands. Therefore, more realistic mesocosm experiments are needed
110 to better understand soil ecosystem responses during and after extreme flood events, and to
111 help develop strategies to minimize the effects of these events on agricultural land.

112 The main aim of this study was to evaluate the impact of different types of extreme flood
113 event (9 weeks) on soil functioning within intact soil mesocosms with and without indigenous
114 vegetation to reflect different possibilities that can occur in nature, in comparison with non-
115 flooded mesocosms. Four different flood typologies were designed to separate the influence
116 of above-ground and below-ground processes (i.e. flooded soil cores with indigenous
117 vegetation versus flooded soil cores without indigenous vegetation) and to simulate the
118 presence or absence of turbid floodwater (i.e. dark versus light conditions). Specifically, we
119 addressed: (i) alterations in element cycling and water quality (Fe, P, N, C) during the
120 extreme flood event (9 weeks) and 5 weeks of soil recovery, air quality (GHG emissions),
121 habitat provision and biological population regulation during 5 weeks after the floodwater
122 was removed (changes in soil microbial community structure, number of earthworms); and
123 (ii) the relation between the cause of these alterations (flood typology), soil parameters and
124 GHG emissions. We hypothesised that (i) flood types in which the availability of light is
125 restricted will result in a greater loss of soil functionality due to negative feedbacks caused by

126 the death of the vegetation, and (ii) a part of the indigenous vegetation will survive when the
127 light is not restricted and will be key to maintaining soil function during and after flooding.
128
129 **2. Materials and methods**
130 *2.1 Soil sampling and soil properties*
131 Sixteen intact soil samples were collected directly from the surface Ah horizon (0-10
132 cm) of a sheep-grazed, *Lolium perenne* L. dominated, low intensity grassland, located in
133 Abergwyngregyn, Gwynedd, North Wales (53º14'21"N, 4º00'57"W) in October 2014. The
134 soil samples, which consisted of 100 cm^3 blocks, were removed intact with associated
135 vegetation (sward height ca. 4 cm). The soil is classified as a sandy clay loam textured Eutric
136 Cambisol with fine crumb structure as detailed in Palomo et al. (2006) and summarized in
137 Table S1. The soil receives an annual fertiliser dose of 100 kg N ha^{-1}, 20 kg K ha^{-1} and 20 kg
138 P ha^{-1}.
139 Soil electrical conductivity (EC) and pH were determined in 1:1 (v/v) soil: distilled
140 H_2O extracts (Smith and Doran, 1996). Calcium carbonate ($CaCO_3$) was determined by the
141 Van Slyke manometric method (Nelson, 1982). Exchangeable cations (Na, K, Ca, Mg and Al)
142 were extracted by shaking (1 h, 20ºC) using a 1:10 (w/v) soil:0.5 M $BaCl_2$ extract with
143 subsequent cation analysis using a Series 720 ICP-OES (Agilent Technologies Inc., Santa
144 Clara, CA). Available P was quantified in a 1:5 (w/v) soil:0.5 M acetic acid extract (1 h, 200
145 rev min^{-1}) with subsequent P analysis by the molybdate blue method of Murphy and Riley
146 (1962). A CHN-2000 analyser (Leco Corp., St Joseph, MI) was used to determine total
147 organic carbon (C) and nitrogen (N) in soil. NO_3^- and NH_4^+ in soil solution were extracted
148 according to Giesler and Lundström (1993) and determined with a Skalar San$^+$ segmented
149 flow analyser (Skalar UK Ltd, York, UK).
150
151 *2.2. Experimental design and treatments*

Immediately after collection from the field, the intact soil blocks (ca. 1 kg) were placed at the bottom of transparent 110 × 80 × 270 mm (l × w × h) polypropylene containers. The top of the containers were left open to facilitate gas exchange. No drainage holes were placed in the base. The containers were transferred to a climate-controlled Fitotron® plant growth chamber (Weiss Technik UK Ltd, Ebbw Vale, UK) with photoperiod of 16 h day^{-1}, light intensity of 350 µmol m^{-2} s^{-1}, temperature of 15ºC and relative humidity of 70 %. A single Rhizon® sampler (Rhizosphere Research Products, Wageningen, Netherlands) was inserted into the centre of each soil block to non-destructively recover soil solution. To label the plant-available P pool, 100 ml of a solution containing ^{33}P ($H_3{}^{33}PO_4$, 111 TBq $mmol^{-1}$; 1.23 kBq ml^{-1}; American Radiolabeled Chemicals Inc., St Louis, MO) was evenly applied to the soil surface of each container two weeks before the flooding started.

The experiment had four distinct stages:

1. Pre-flood stage: This initial phase involved placing each plant-soil mesocosm (n = 16) into the growth chamber for 2 weeks to allow acclimation. The soils were maintained field-moist by the daily addition of oligotrophic river water (collected from the Aber River adjacent to where the soil samples were collected) based on their weight loss. This also permitted ^{33}P to become re-distributed within the plant-microbial-soil system;

2. Flood stage: This phase incorporated the four main flood treatments alongside an unflooded control treatment (maintained at field-moist based on weight loss), namely:

T_1: flooded soil with no above-ground vegetation and maintained in the dark [flood+dark (no veg.) treatment]

T_2: flooded soil with above-ground vegetation and maintained in the dark (flood+dark treatment)

T_3: flooded above-ground vegetation (from T_1) with no soil and maintained in the dark [flood+dark (only veg.) treatment]

177 T_4: flooded soil with above-ground vegetation and maintained in the light (flood+light
178 treatment)
179 T_5: unflooded soil with above-ground vegetation and maintained in the light
180 (control+light treatment)
181 Firstly, the vegetation from 4 replicate containers was cut at ground level (T_1) and the
182 grass clippings transferred to 4 new containers containing no soil (T_3), increasing the number
183 of containers up to 20. Secondly, river water was used to flood 16 of the containers (T_1-T_4),
184 including the four in which the grass was cut to soil level, and the four containing grass only.
185 Each container received ca. 1200 ml of river water to achieve a flood height of 10 cm above
186 the soil surface (reflecting typical flood depths observed in the region during extreme weather
187 events; Figs. S1-S3). Twelve containers were subsequently covered by black plastic prevent
188 light entry into the microcosms (T_1-T_3). Four microcosms were left unflooded and exposed to
189 the light as a control (T_5). The floodwater was maintained at a constant height throughout the
190 experiment through the addition of river water, while the unflooded control treatments were
191 maintained field-moist as in the pre-flood phase. This phase lasted for 9 weeks.
192 *3. Soil recovery stage:* At this point in the experiment the flood water was carefully
193 removed from treatments T_1-T_4. All treatments were then exposed to the light and ambient air
194 and allowed to dry out naturally. This stage had a duration of 5 weeks and aimed to simulate
195 the period before which agronomic practices recommenced.
196 *4. Soil functions assessment stage:* To evaluate any legacy effects of the flooding on
197 soil functioning, a pot-based bioassay was performed. Briefly, 500 g of soil was recovered
198 from each treatment (with roots removed) and placed in a 500 cm^3 pot and sown with two 3 d-
199 old maize seedlings (*Zea mays* L.). The pots were placed in the same growth chamber as used
200 above for 28 d, except that the temperature was maintained at 20 ºC. Thirty ml of a solution
201 containing N, P and K (10 mM KNO_3, 15 mM KH_2PO_4) were applied on a weekly basis to
202 remove nutrient limitation. After 7 d, one plant from each pot was removed. The pots were

203 weighed and watered with river water 3 times per week to maintain the soils in a field-moist
204 condition.
205
206 *2.3. Measurement of soil chemical and biological indicators*
207 *2.3.1. Element cycling and water quality*
208 Depending upon treatment and experimental stage, soil solution and floodwaters were
209 collected approximately weekly and analysed for pH, electrical conductivity (EC), total Fe
210 (Loeppert and Inskeep, 1996), Fe^{2+} (Loeppert and Inskeep, 1996), P (Murphy and Riley,
211 1962), ^{33}P, NO_3^- (Miranda et al., 2001), NH_4^+ (Mulvaney, 1996), total dissolved N (TDN) and
212 dissolved organic C (DOC). Additional samples were taken the day before and after the first
213 and the last days of the flood stage. Fe, P, NO_3^- and NH_4^+ were determined by
214 spectrophotometry on a PowerWave XS microplate reader (BioTek Instruments Inc.,
215 Winooski, VT). TDN and DOC were determined using a Multi N/C 2100/2100 analyser
216 (AnalytikJena AG, Jena, Germany). Dissolved organic N (DON) was calculated by
217 subtraction of NO_3^- and NH_4^+ from the TDN value. ^{33}P in solution was determined by liquid
218 scintillation counting on a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes,
219 UK) after mixing with Optiphase Hisafe 3 scintillation fluid (PerkinElmer Inc., Waltham,
220 MA). Soil redox potential was determined periodically through the experiment using a
221 SenTix® probe (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim,
222 Germany) inserted in the surface of the soil (0-3 cm depth).
223 During the flood stage, the total amount of nutrient released into the soil solution and
224 overlying floodwater ($C_{release}$) was calculated as follows:
225 $$C_{release}\ (\text{mg container}^{-1}) = [C_{sol} \times V_{soil} \times \Theta] + [C_{flood} \times V_{flood}] \quad \text{(Eqn. 1)}$$
226 where C_{sol} and C_{flood} are the concentration of nutrient in the soil solution and floodwater
227 respectively, V_{soil} and V_{flood} are the volume of soil and floodwater respectively and Θ is the
228 volumetric water content (0.5 $cm^3\ cm^{-3}$).

229

230 *2.3.2. Air quality*

231 Although some small N_2O emissions from residual soil NO_3^- at the start of flooding
232 were detected in a similar previous experiment (Sánchez-Rodríguez et al., 2017), the most
233 important emissions were measured during the soil recovery phase (Sánchez-Rodríguez et al.,
234 2017, 2018). This is the reason why we focused our GHGs measurements on the soil recovery
235 stage. On the last day of the flood stage and through the flood recovery stage the mesocosms
236 were hermetically sealed during the samplings (the day before the floodwater was removed,
237 the same day after the floodwater was removed and weekly until the end of the soil recovery).
238 At 0 h and 1 h after sealing (between 10.00 h and 12.00 h), 20 ml of headspace gas was
239 removed using a hypodermic syringe via a rubber septum placed in the mesocosm lid, and the
240 gas transferred to a pre-evacuated glass vial. CH_4, CO_2 and N_2O concentrations in the vials
241 were subsequently analysed by gas chromatography using a Clarus 500 GC equipped with a
242 HS-40Turbomatrix headspace analyser, ^{63}Ni electron-capture detector and flame ionization
243 detector connected to a methanizer (PerkinElmer Inc.). Fluxes were estimated as the
244 difference in gas concentration at time 0 and 1 h and after correction for both temperature and
245 the ratio between chamber volume and soil surface area (MacKenzie et al., 1998). Cumulative
246 fluxes were estimated by linear interpolation, multiplying the mean of two successive daily
247 fluxes by the number of hours between the two measurements and adding that amount to the
248 previous cumulative total.

249 *2.3.3. Habitat provision and biological population regulation*

250 At the end of the soil recovery stage (second phase of the experiment), the dry weight of grass
251 and number of earthworms per box were weighed and counted, respectively. Maize plant
252 height was measured after 14 and 28 d, plant dry weight was determined (80 ºC, 72 h) and
253 mineral element concentrations in above- and below-ground biomass determined by ICP-OES
254 after dry-ashing and digestion with hydrochloric acid (Adrian, 1973) at the end of the soil

functions assessment stage (fourth phase of the experiment), To evaluate changes in microbial community structure, soil samples (25 g) from each container were collected at the beginning and at the end of soil recovery stage and stored at -80°C for PLFA analysis and subsequently determined as described in Bartelt-Ryser et al. (2005). Although a total of 50 fatty acids were identified in the soil samples, Table 1 shows the 23 with a concentration higher than 0.5% of the total PLFAs that were used as biomarkers for the different taxonomic groups according to Ratledge and Wilkinson (1988), Bedard and Knowles (1989), Bowman et al. (1991, 1993), Kieft et al. (1994), Paul and Clark (1996), Bossio and Scow (1998), Olsson et al. (1999), Zelles (1999), Niklaus et al. (2003), Bartelt-Ryser et al. (2005) and using standard nomenclature as described in Frostegård et al. (1993). Despite their known limitations (Frostegård, 2011), the following PLFA biomarker ratios were calculated: fungi-to-bacteria as an indicator of large scale community shifts, predator-to-prey (protozoa/bacteria) to estimate the availability of nutrients to support higher trophic levels, Gram+-to-Gram- as an indicator of soil aeration state (Bossio and Scow, 1998), saturated-to-unsaturated fatty acids (sat/unsat) as an indicator of the stability of the microbial community, mono-to-polyunsaturated fatty acids (mono/poly), precursor-to-cyclopropane fatty acids (precursor/cyclopropane fatty acids; 16ω/17 cyclo and 18ω/19 cyclo) as indicators of high stress (Knivett and Cullen, 1965).

2.4. Statistical analysis

Repeated measured analysis of variance (RM of ANOVA) based on a completely randomized design with 4 treatments and 4 replications per treatment were applied to pH, EC, Fe, P, ^{33}P, NH_4^+, NO_3^-, DON, DOC in soil solution [flood+dark (no veg.), flood+dark, flood+light and control+light] and flood water [flood+dark (no veg.), flood+dark, flood+dark (only veg.) and flood+light] as well as for soil redox potential and daily GHG emissions during the soil recovery stage [flood+dark (no veg.), flood+dark, flood+light and control+light]. Bonferroni multiple comparison test at a probability level of 0.05 was used to

identify differences between treatments. ^{33}P (‰) is expressed as the ^{33}P of the sample / ^{33}P of the original radioactive solution (accounting for radioactive decay) × 1000 for soil solution samples and, additionally, multiplying by 13 only in the case of floodwater samples, because the initial 100 ml of radioactive solution (applied to the soil of all containers) were diluted when 1.2 l of fresh water were applied to flood+dark (no veg.), flood+dark and flood+light containers at the beginning of the flood-stage.

Analysis of variance (ANOVA) based on a completely randomized design with the same number of treatments and replications was applied to the plant biomass, plant nutrient content, earthworm, cumulative GHG, and PLFA data. In these cases, Tukey's HSD *post hoc* was used to identify treatment differences.

To identify relationships between soil microbial communities and GHG daily fluxes, soil redox potential, NO_3^- and NH_4^+ in soil solution, and to find differences between treatments, principal component analysis (PCA) was performed at the end of the flood and soil recovery stages, based on a data correlation matrix to elucidate differences between treatments. Finally, Pearson correlations were carried out between these parameters using the data obtained during the soil recovery. The statistical analyses were performed using the statistical package SPSS software v22.0 (IBM Inc., Armonk, NY).

3. Results

3.1. Element cycling and water quality: Response of soil chemical indicators to extreme flooding

Few major treatment effects were observed in soil solution pH, however, slightly higher soil solution pH values were obtained in the flood treatments [flood+dark(no veg.), flood+dark and flood+light] relative to the control+light treatment 3-5 weeks after flooding (Fig. 1a). The floodwater (Fig. 1b), pH values were similar to those of soil, with the exception of the flood+light treatment (T_4) which had a significantly higher pH relative to the dark-flood

307 treatments (T_1 and T_2).In contrast to pH, major changes in soil EC and redox potential in
308 response to flooding were observed (Fig. 1c-e). In all flood treatments, soil solution EC
309 increased markedly during the experiment, especially during the flood stage, but quickly
310 declined at the soil recovery stage (Fig. 1c). The increase in EC was slower in the illuminated
311 flood treatment (T_4) relative to those maintained in the dark. In comparison to the soil, the EC
312 of the floodwater remained lower with the highest values seen in the treatments containing
313 soil and maintained in the dark (Fig. 1d). Redox potential rapidly declined in the flood
314 treatments and then remained constant throughout the flood period (Fig. 1e). The redox
315 potential remained consistently higher in the illuminated flood treatments in comparison to
316 those maintained in the dark. After flood removal, the redox potential rapidly increased,
317 reaching positive values again within ca. 7 d and values close to the non-flooded treatment
318 control (T_5) by 21 d.

319 The dynamics of Fe^{2+} (not shown) in soil solution and floodwater exhibited a similar
320 temporal pattern to total Fe (Fig. 2ab) and represented 60% of the total Fe in soil solution and
321 65% of total Fe in flood water (averaged across the whole flooding period). Fe concentrations
322 in soil solution rapidly increased after commencement of the flood treatment (albeit slower in
323 T_4 relative to T_1 and T_2), peaking at ≈29 mg Fe l^{-1} at the end of flood stage (Fig. 2a). In
324 comparison, soil solution Fe concentrations in the control treatment (T_5) remained low
325 throughout the experiment. After flood removal, soil solution Fe in the flooded treatments
326 rapidly dropped to values similar to the control. Significant amounts of Fe accumulated in
327 floodwater albeit at lower concentration that observed in soil solution (Fig. 2b). Floodwater
328 Fe concentrations in the flooded treatments containing soil started to increase after 7 d,
329 reaching maximal values by week 4. In contrast, Fe concentrations remained very low when
330 no soil and only vegetation was present (T_3). The potential Fe lost (mg $mesocosm^{-1}$) was 14.8
331 ± 0.4 in T_1 [flood+dark (only veg.), 14.9 ± 2.0 in T_2 (flood+dark) and 12.3 ± 0.6 in T_4
332 (flood+light), being 0.5 ± 0.1 from the grass in T_3 [flood+dark (only veg.)].

Phosphorus in soil solution remained low throughout the experiment (0.10 to 0.54 mg l^{-1}). Although a simple explanation is not possible, several spikes in soluble P were seen during the flood stage, however, few significant treatment effects were observed (Fig. 2c). This is supported by the data obtained for ^{33}P which also showed a very low P concentration and no significant differences between treatments (Fig. 2e). After flood removal, the amount of ^{33}P in soil solution was negligible (<0.1 ‰ of the initial value). In contrast to soil solution, significant increases in both P and ^{33}P were observed in floodwater (Fig. 2df). Floodwater P concentrations were highest in treatments containing no soil (T_3, grass only) and significantly lower in the illuminated flood treatment (T_4). The calculated potential P loss from the soil (mg mesocosm^{-1}) was 0.6 ± 0.2 in T_1 [flood+dark (no veg.)], 0.8 ± 0.2 in T_2 (flood+dark) and 0.2 ± 0.1 in T_4 (flood+light). This value was higher for T_3 (grass only) being 1.3 ± 0.2 mg mesocosm^{-1}.

A similar pattern to Fe was observed for NH_4^+ during flooding (Fig. 3a). In the control treatment (T_5), NH_4^+ concentrations remained low throughout the experiment (<0.7 mg $l^{-1)}$. The imposition of a flood, however, induced a progressive increase in NH_4^+ concentration until the end of the flood stage. This was particularly evident in the flooded treatments without light (T_1, T_2) with NH_4^+ concentrations increasing immediately after flooding. When light was present (T_4), a significant lag phase in NH_4^+ production occurred after flooding with the concentrations being much less than in the dark treatments. After flood removal, the NH_4^+ concentrations initially fell in all treatments, however, this was greater when plants were present in the mesocosms. NH_4^+ was only detected in the overlying floodwater in the dark treatments (Fig. 3b). The calculated potential NH_4^+ lost (mg mesocosm^{-1}) was considerably higher in T_1 [16.9 ± 2.0, dark+flood (no veg.)] and T_2 (15.4 ± 1.0, dark+flood) than in T_3 (2.7 ± 1.3, dark+flood [only veg.)] and T_4 (4.3 ± 2.0, light+flood).

The concentration of NO_3^- in soil solution was low in all treatments throughout the flood stage (Fig. 3c). However, upon flood removal significant increases in NO_3^- were

observed, especially in those mesocosms which had been previously maintained in the dark. In comparison to NH_4^+, the levels of NO_3^- in floodwater were extremely low, however, significantly more was NO_3^- observed in the dark-flood treatments (Fig. 3d). Across all treatments, the loss of NO_3^- from the mesocosms was very low in the flooded treatments (<1.1 mg mesocosm^{-1}).

Overall, flooding induced both an increase in DON and DOC concentrations in soil solution over time relative to the unflooded control (Fig. 4c). Concentrations tended to be higher in the dark treatments. Once the floodwater had been removed, DOC concentrations rapidly declined within 14 d, however, this decline was considerably slower for DON. Levels of DOC in floodwater increased over time, however, the dynamics and treatment effect were different to those seen in soil solution (Fig. 4d). In contrast, levels of DON in floodwater remained low with few treatments effects apparent (Fig. 4b). The potential DON lost (mg mesocosm^{-1}) was 28.3 ± 2.4 in T_1, 27.5 ± 0.6 in T_2 and 14.7 ± 0.8 in T_4 and the potential DOC lost (mg mesocosm^{-1}) was 362 ± 19 in T_1, 352 ± 20 in T_2 and 339 ± 12 in T_4.

3.2. Air quality: Greenhouse gas emissions

Figure 5 shows the daily production and cumulative fluxes of CH_4, CO_2 and N_2O from the last day of the flood stage and throughout the soil recovery stage. Daily CH_4 fluxes were greatest at the end of flooding and rapidly declined after floodwater removal reaching fluxes close to background 20 d after flood removal, especially for T_1 and T_2 (more constant for T_4, flooding in the light). The highest cumulative CH_4 fluxes (P = 0.003) were found for treatment including floods (T_1 > T_2 > T_4 > T_5; Fig. 5b). Daily CO_2 fluxes in the dark treatments were initially higher than the illuminated treatments both at the end of the flood stage and during soil recovery. This trend subsequently reversed after 2 weeks of soil recovery. Although the dynamics of CO_2 production differed between treatments, the cumulative amount of CO_2 produced over the recovery phase was similar (Fig. 5d). Daily

385 N_2O fluxes were highest in the darkened flood treatments (T_1 and T_2) and showed a
386 progressive production throughout the flood recovery stage (Fig. 5ef). When light was present
387 in the flood stage then almost no N_2O was produced, and responded in a near-identical way to
388 the unflooded control.
389
390 *3.3. Habitat provision and biological population regulation*
391 *3.3.1 Vegetation responses to extreme flooding*
392 As expected, the fertile agricultural Eutric Cambisol had a high base saturation, high
393 available P content, nitrification potential (NO_3^- > NH_4^+; Table S1) and supported rapid
394 sward growth in the absence of flooding. After imposing the flood treatments and in the
395 absence of light, the vegetation was able to survive for approximately 3-4 weeks, however,
396 beyond this point, senescence and subsequent decomposition of the above-ground biomass
397 occurred (T_2 and T_3). Little recovery of this grass occurred in the subsequent recovery period
398 when the floodwater was removed and the soil allowed to dry out (Table 2). In contrast, when
399 light was present (T_4), a considerable amount of the above-ground vegetation was able to
400 survive throughout the flood period and readily recovered after floodwater removal. Despite
401 this sward recovery, grass production in the flooded treatments was less than produced in the
402 unflooded control (T_5).
403 No significant differences were observed for plant height, dry weight and mineral
404 nutrient concentration in the maize crop in the bioassay performed at the end of the
405 experiment (fourth phase). The only notable exceptions to this were the reduced growth for
406 plants grown in the T_1 [dark+flood (no veg.)] soil (SI, Table S2) and minimum alterations in
407 foliar C and K (SI, Table S3).
408
409
410 *3.3.2. Soil mesofauna*

411 Abundant earthworms were present in the soil at the start of the experiment. At the end
412 of the experiment, however, live earthworms could only be recovered from the soil of the
413 unflooded controls (Table 2).
414
415 *3.3.3. Soil microbial biomass and community structure*
416 Microbial PLFA analysis after the flood phase (Fig. 6a) and after soil recovery (Fig.
417 6b) showed that flooding induced a significant reduction in biomass relative to the unflooded
418 control. Microbial community structure was also significantly altered by flooding (Fig. 6cd).
419 Overall, flooding significantly decreased the amount of PLFAs indicative of protozoa (P =
420 0.014 and P = 0.002), putative arbuscular mycorrhizas (P = 0.004 and P = 0.035) and fungi (P
421 < 0.001 and P < 0.001), and increased the amount of Gram+ bacteria (P < 0.001 and P =
422 0.005) and actinomycetes (P = 0.111 and P = 0.047) in comparison with the unflooded control
423 (after flood and soil recovery stages, respectively, in each case). The PLFA ratios were also
424 altered by flooding, reducing fungi/bacteria (P < 0.001 and P < 0.001), predator/prey (P =
425 0.012 and P = 0.001) and 18w/19cyclo (P = 0.043 and P = 0.020) but increasing
426 Gram+/Gram− (P = 0.007 and P = 0.032), sat/unsat (P < 0.001 and P = 0.033) and mono/poly
427 (P < 0.001 and P = 0.002) (Fig. 6ef).
428 Figure 7 shows the relationships in PLFA between the treatments after the flood and
429 soil recovery stages, i.e. taxonomic groups (Fig. 7ab) and fatty acids ratios (Fig. 7cd). These
430 PCAs show clear separation between the control and flood treatments with the absence of
431 light exacerbating the differences relative to the flooded treatment maintained in the light. At
432 the end of the flood stage, the first three components of the PCA (Fig. 7a) explained 75.2% of
433 the total variance. The first principal component (PC1) with a high loading for fungi, redox,
434 protozoa, Gram+ and NH_4^+ accounted for 47.3%; the PC2 that explained 13% of the variance
435 (PC2) had a high loading for NO_3^- and N_2O. Some of the correlations of the variables used to
436 do the PCA were: fungi-redox (r = 0.87, P < 0.001), fungi-NH_4^+ (r = −0.89, P < 0.001), fungi-

437 Gram+ ($r = -0.89$, $P < 0.001$), fungi-CH_4 ($r = -0.61$, $P = 0.006$), redox-Gram+ ($r = -0.89$, $P <$
438 0.001), and protozoa-Gram+ ($r = -0.86$, $P < 0.001$).

439 At the end of the soil recovery stage (Fig. 7b), PC1 had a high loading for Gram+,
440 actinomycetes (actino), fungi and anaerobic bacteria and explained 40% of the total variance
441 while PC2 explained 21.2%, with a high loading for Gram− bacteria followed by redox (79%
442 of total variance explained by the first 3 components). See Supp. Info for further information
443 relating to Fig. 7cd.

444 Table 3 shows the correlation matrix between GHG daily fluxes and soil parameters
445 during soil recovery stage. CH_4 emissions were related with anaerobic conditions (low redox
446 potential, high NH_4^+ concentrations). Lastly, soil redox potential was negatively correlated to
447 NH_4^+ in soil solution (Table 3).

448 Additional correlations between PLFAs and the most abundant fatty acids (> 2% of
449 the total PLFAs) and GHG daily fluxes, redox potential in soil, NH_4^+ and NO_3^- are shown in
450 Table 4. CH_4 emissions were negatively correlated with PLFAs and the majority of these fatty
451 acids after the flood stage only. These negative correlations also occurred for NH_4^+ after both
452 stages. CO_2 emission was negatively correlated with total PLFAs and many individual fatty
453 acids after the flooding stage but were positively related after the soil recovery stage (Table
454 4). As expected, redox potential in the soil was positively correlated with PLFAs in both
455 stages, indicating a higher microbial activity in the soil when redox potential is positive.

456

457 **4. Discussion**

458 This study clearly demonstrated that when soils with no previous history of flooding
459 are subjected to intense waterlogging, major changes in soil functions and ecosystem service
460 delivery occur. The simulated flood typologies reflect those seen recently in the UK and
461 elsewhere around the world (Dodds, 2014; Hai et al., 2017; Romshoo et al., 2018).

462 *4.1. Habitat provision: Primary productivity and earthworm abundance*

As expected, the vegetation in our mesocosms was heavily affected by long-term flooding, especially when light was restricted (Mommer et al., 2005a, b; Das et al., 2009). This suggests that alongside flood duration (Fig. S1-S4), the typology of the flood event is also critical in determining the likelihood of vegetation survival. Extreme flooding can be associated with large amounts of turbid sediment in the overlying water which restricts light penetration (Fig. S3), whilst other flood events are associated with groundwater rise and relatively clear overlying waters (Fig. S4). In recent years, extreme flood events in the UK have been associated with large amounts of sediment in the floodwater which remains suspended for long time periods via wind-mediated turbulence (Fig. S5). The impact of light restriction and sediment load therefore requires greater consideration in future studies on the impact of flooding on plant-soil systems (Squires et al., 2002). It is also clear from our study that the presence of light favoured a more rapid recovery of grass production 5 weeks after floodwater removal, however, no significant differences were observed in maize sown after the soil recovery period in any treatment, highlighting the capacity of the soil to self-ameliorate.

Earthworms perform vital functions in grasslands including the promotion of soil organic matter turnover, nutrient recycling, aeration, drainage and the amelioration of compaction (Langmaack et al., 1999). All of these attributes are frequently linked to greater primary productivity. A decline in earthworm abundance, diversity or activity can therefore be viewed as a severe loss of soil quality (Coyle et al., 2017). In our experiments, long-term flooding resulted in a complete loss of the earthworm population with no recovery (i.e. hatching from cocoons) observed 5 weeks after floodwater removal. This is in general agreement with Ivask et al. (2012) who suggested that earthworm populations were particularly affected by long term flooding. The loss of earthworms from all flooding treatments in comparison with the unflooded controls could have potential short and long-term effects on soil functionality (e.g. the loss of fertility, and a decline in soil structure).

489

490 *4.2. Impact of flooding on element cycling, water and air quality*

491 In our study, flooding increased soil pH, consistent with previous studies in rice paddy
492 soils and salt marshes by Ponnamperuma (1972) and Negrin et al. (2011), however, the
493 overall effect was small. We conclude that this is unlikely to cause lasting changes in nutrient
494 bioavailability or explain our flood-induced shifts in microbial diversity. A clear negative
495 effect of flooding on EC was apparent, however, the levels were insufficient to induce
496 osmotic stress in roots. We attribute the increase in EC to the release of mineral elements
497 from soil and during vegetation senescence (e.g. Fe, P, NH_4^+).

498 Flooding significantly altered the redox conditions in the soil, with all flood treatments
499 rapidly becoming anaerobic (Reddy and De Laune, 2008). However, the vegetation in T_4 kept
500 redox values higher than in T_1 and T_2 (without vegetation and in which vegetation died after
501 3-4 weeks of flood, respectively), probably because the plants were able to deliver oxygen to
502 the rhizosphere, alleviating the anaerobic conditions to some extent. Although this effect has
503 been observed for plant species such as *Spartina alterniflora* (Colmer, 2003), other authors
504 found no influence of vegetation on redox potential in soil with highly reduced conditions
505 (Negrin et al., 2011). This supports the tenet that selection of grass varieties with greater
506 potential for aerenchyma formation will offer greater protection against long term flooding
507 (de Souza et al., 2017).

508 The different element cycles (partially) assessed in this study were altered
509 considerably. There was a clear effect of flooding on Fe release (soil solution and floodwater),
510 however, a less clear pattern was apparent for P because the increase of P was only observed
511 in the floodwater of T_2 and T_3, treatments with vegetation and under light restrictions. We had
512 hypothesized that P held on the surface of Fe-oxyhydroxides would be released under
513 reducing conditions and would migrate upwards to the overlying floodwater where it might
514 stimulate algal production (Nanzyo et al., 2004; Heiberg et al., 2010), however, this was not

515 apparent. This could be due to any P released being reabsorbed back onto Al-hydroxides or
516 being immobilized by the microbial biomass. The evidence from the ^{33}P tracers also indicated
517 that the majority of solubilised P in the floodwater was actually derived from the
518 decomposing grass. Based on our field observations, it is also possible that Fe-P minerals may
519 have formed directly at the soil surface where the conditions are less anoxic (Lindsay et al.,
520 1989; Roden and Edmonds, 1997). The potential for P and Fe redistribution in soil during
521 flooding therefore warrants further research.

522 We ascribe the net accumulation of NH_4^+ during flooding to the mineralization of soil
523 organic matter (T_1), the necrosis of plant tissues and an inhibition of nitrification (Nielsen et
524 al., 1996). In the case of the soil, we cannot distinguish between N released from microbial
525 processes or from autolysis of live roots and subsequent excretion of NH_4^+ (Marella et al.,
526 2017). The low C:N ratio of earthworms (ca. 3:1) and vegetation (ca. 24:1) is likely to favour
527 net NH_4^+ release during their decomposition (Zheng and Marschner, 2017). In addition, when
528 photosynthesis is restricted and C for plant respiration becomes limited, root and shoot
529 autolysis will commence leading to NH_4^+ excretion to the external medium (i.e. during
530 proteolysis and creation of organic/keto acids; Marella et al., 2017). This operation of this
531 pathway is supported by the greater release of soluble N in treatments where light was
532 restricted. The NH_4^+ concentration in floodwater of T_2 during the flood stage was
533 approximately the sum of the concentrations of T_1 (soil without above-ground vegetation) and
534 T_3 (vegetation from T_1) treatments (Fig. 3b). This suggests that 35-40 % of the NH_4^+
535 originated from the above-ground vegetation and 60-65 % from the soil compartment.

536 In addition to plant uptake, losses of NH_4^+ via NH_3 volatilization (not determined in
537 this experiment) could also have occurred under inundation (Zhong-Cheng et al., 2012; Chen
538 et al., 2015). The high pH of the floodwater (pH 7.0-8.5) would favour this loss pathway and
539 could help explain the decrease in NH_4^+ for T_1, T_2 and T_3 in the overlying water at the end of
540 the flood stage. During soil recovery, the soil water content of T_1, T_2 and T_4 decreased,

541 resulting in re-aeration of the soil, re-establishment of nitrification, leading to a decrease in
542 NH_4^+, and increases in NO_3^- and N_2O production.

543 Significant amounts of DOC and DON accumulated in the soil and overlying water
544 during flooding. This can be attributed to the release of organic compounds from organisms
545 killed by either (i) a lack of O_2 (e.g. mesofauna), (ii) osmotic shock, or (iii) metal toxicity
546 (e.g. by Fe^{2+}, Mn^{2+}) (Kieft et al., 1987, 1994; Denef et al., 2001; Fierer and Schimel, 2003).
547 Based on the very high DOC-to-DON ratio (>70:1), however, we hypothesize that this C is
548 mainly derived from anaerobic respiration by-products (e.g. ethanol, organic acids) excreted
549 by plants and microbes into the external medium (Jones et al., 2009). Although soluble C
550 could also be released during the reduction and solubilisation of Fe-oxyhydroxides, this is not
551 favoured based on the DOC-to-DON ratio of soluble C held on the exchange surfaces of this
552 soil (ca. 12:1; Jones and Willett, 2006). The measured decrease in DOC concentration
553 following the removal of floodwater is most probably related to the removal of O_2 limitation
554 and a stimulation of microbial activity (Frank et al., 2014).

555 Although we used minimally disturbed blocks of vegetated soil in our mesocosms,
556 some aspects of real flood events could not be replicated. For example, in our experiment
557 there was no water turbulence and no erosional loss of soil, and the soil blocks were only 10
558 cm deep. While this reflects the main rooting zone, our results cannot be extrapolated easily
559 to subsoils where the C content and root density is much lower, and the effects of flooding
560 may be less severe. However, the nutrient losses due to the different flood typologies—
561 aggravated under light restriction—indicate the importance of considering the origin of the
562 floodwater, i.e. whether it contains suspended particles.

563 Alterations in the assessed gaseous emissions from the flooded mesocosms were also
564 dependent on flood typology. Normally, very low emissions of CO_2, CH_4 and N_2O have been
565 observed during flooding in previous experiments with this soil (Sánchez-Rodríguez et al.,
566 2017). CH_4 emissions were, however, detected immediately after floodwater removal. We

ascribe this to the release of CH_4 produced during flooding, but which had become trapped within the soil pores until floodwater removal (Moore and Roulet, 1993; Sánchez-Rodríguez et al., 2017). In our experiment, the prevailing conditions under flooding (-100 mV redox potential, high DOC, senescing vegetation) were ideal for CH_4 production (Hou et al., 2000). This was most apparent in the dark treatments where plant senescence was greatest. As oxygen was introduced back into the soil after flooding, and as alternative electron acceptors became available (e.g. NO_3^-), the rate of CH_4 emissions quickly decreased (Yuan et al., 2008). This might also have been facilitated by an increase in CH_4 oxidation within the soil (Zhang et al., 2012).

At the start of the soil recovery stage, the soil solution in the flooded treatments (T_1, T_2 especially, and T_4) had high concentrations of labile N and C (i.e. NH_4^+, DON and DOC). In addition, the soil redox potential increased, favouring conditions to produce N_2O (Hou et al., 2000; Kim et al., 2010) as an intermediate product of nitrification. The observed decrease in soil solution NO_3^- concentration during the soil recovery stage also indicates losses via denitrification. However, the recovering vegetation under non-light restrictions could also have acted as a NO_3^- sink, contributing to the daily and cumulative fluxes of N_2O, being significantly lower for T_4 (flood+light) and T_5 or control in comparison with T_1 and T_2 (flood+dark without and with vegetation, respectively). Again, light restriction (flood typology) and the presence of grass were essential to understand gaseous C and N losses. Finally, it should be mentioned that, the limited depth of the mesocosms (10 cm) could have underestimated the gas fluxes measured during the 5 weeks after the floodwater removal as compared to field conditions but further research is needed to confirm this.

4.3. Microbial biodiversity: Biological population regulation

We present clear evidence that the microbial community was significantly affected by prolonged flooding, the presence of vegetation and time since flooding. However, in some

593 flooding situations, water percolates through the soil in either an upward (groundwater
594 flooding) or downward (surface water flooding) direction. This mass flow may remove
595 microbial end-products and also change the redox status of the soil in comparison to our
596 mesocosms where no mass flow occurred.

597 Our results are in general agreement with Ferré et al. (2012) who found a higher
598 Gram+/Gram− bacteria ratio in flooded soils, probably as Gram+ bacteria (branched fatty
599 acids) are believed to be more stress tolerant than Gram− bacteria (monounsaturated fatty
600 acids). In addition, our results are in line with Reichardt et al. (2001) who found higher
601 concentrations of fungi in non-flooded conditions in comparison with flooded soils. In most
602 cases, however, the change in the individual amount of PLFAs was small in the different
603 treatments. However, it should be noted that this mainly reflects changes in the active
604 microbial biomass which may only represent <10% of the total PLFA in soil. The impact of
605 these changes in community structure on soil functioning remain uncertain due to the large
606 functional redundancy that exists in soil. What is clear, however, is that it had little impact on
607 plant growth and soil performance in our maize bioassay undertaken at the end of the
608 experiment. Further, in comparison to the loss of earthworms we expect that small shifts in
609 microbial community structure are of less importance in the longer term.

610 Other PLFA ratios were affected by the prolonged flooding treatments, for example
611 the ratio of sat/unsat agrees with the increase in saturated fatty acids under flooding described
612 in Bossio and Scow (1998), and 16w/17 cyclo and 18w/19 cyclo ratios that are related with
613 stress (Knivett and Cullen, 1965), probably due to low O_2 availability in the flooded
614 treatments. The detection of 16:1w7c and 18:1w9c fatty acids in soil taken at the end of the
615 flood period supports the presence of methanotrophs (Bedard and Knowles, 1989; Bowman et
616 al., 1991, 1993).

617 Lastly, the role of surviving vegetation in T_4 (flood+light, that facilitated less extreme
618 flood conditions) produced an intermediate PLFA profile between T_1-T_2 (flood under

619 darkness without and with soil, respectively) and T_5 (unflooded controls; Figs. 6, 7). The
620 oxygenation of the rhizosphere by living roots and a higher potential redox in T_4
621 (flood+light), probably lessened the impact of flooding and facilitated a quicker recovery of
622 the microbial community.
623
624 **5. Conclusions**
625 Prolonged flood events were shown to induce major shifts in the size and structure of
626 the soil microbial community that led to a decrease in air quality (higher net GHG emissions;
627 CH_4, N_2O) and major alterations in soil biogeochemical cycling as a function of the flood
628 typology. Prolonged flooding in which light is restricted increased the severity of the damage
629 in terms of potential nutrient losses, GHG emissions, soil microbial communities, grass
630 production and speed of recovery, highlighting the key role of the vegetation in maintaining
631 grassland soil functioning. We demonstrated that the decomposition of vegetation is an
632 important source of P loss, especially in flood typologies in which light is restricted where its
633 contribution can be as important as soil in terms of P loss rates. Our results also suggest that
634 anoxia-tolerant vegetation may play a key role in ameliorating the negative effects of flooding
635 on habitat provision, element cycling, and biological population regulation.
636
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Figure legends

Fig. 1 Time course (mean value) of pH and EC in soil solution and flood water, and redox potential in soil, as a function of flood treatments. Vertical bars in the upper part represent Bonferroni values at $\alpha = 0.05$ and the presence of asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). T1: soil samples without vegetation, flooded and kept under darkness. T2: soil samples with vegetation, flooded and kept under darkness. T3: vegetation cut from T1 before the flood stage, flooded and under kept under darkness. T4: soil samples with vegetation, flooded and maintained in the light. T5 (control): soil samples with vegetation and maintained in the light. Four replicates per treatment.

Fig. 2 Time course (mean value) of Fe, P and ^{33}P in soil solution and flood water as a function of flood treatments. Vertical bars in the upper part represent Bonferroni values at $\alpha = 0.05$ and the presence of asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). T1: soil samples without vegetation, flooded and kept under darkness. T2: soil samples with vegetation, flooded and kept under darkness. T3: vegetation cut from T1 before the flood stage, flooded and under kept under darkness. T4: soil samples with vegetation, flooded and maintained in the light. T5 (control): soil samples with vegetation and maintained in the light. Four replicates per treatment.

922 **Fig. 3** Time course (mean value) of NH_4^+ and NO_3^- in soil solution and flood water as a
923 function of flood treatments. Vertical bars in the upper part represent Bonferroni values at α =
924 0.05 and the presence of asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$,
925 ***: $P < 0.001$). T1: soil samples without vegetation, flooded and kept under darkness. T2:
926 soil samples with vegetation, flooded and kept under darkness. T3: vegetation cut from T1
927 before the flood stage, flooded and under kept under darkness. T4: soil samples with
928 vegetation, flooded and maintained in the light. T5 (control): soil samples with vegetation and
929 maintained in the light. Four replicates per treatment.
930
931 **Fig. 4** Time course (mean value) of DON and DOC in soil solution and flood water as a
932 function of flood treatments. Vertical bars in the upper part represent Bonferroni values at α =
933 0.05 and the presence of asterisk/s indicate significant differences (*: $P < 0.05$, **: $P < 0.01$,
934 ***: $P < 0.001$). T1: soil samples without vegetation, flooded and kept under darkness. T2:
935 soil samples with vegetation, flooded and kept under darkness. T3: vegetation cut from T1
936 before the flood stage, flooded and under kept under darkness. T4: soil samples with
937 vegetation, flooded and maintained in the light. T5 (control): soil samples with vegetation and
938 maintained in the light. Four replicates per treatment.
939
940 **Fig. 5** Daily (left) and cumulative (right) fluxes of CH_4, CO_2 and N_2O (mean value) during
941 the soil recovery stage as a function of flood treatments. Different letters mean differences
942 according to Bonferroni multiple comparison test for daily fluxes and Tukey's HSD test for
943 the last determination of cumulative fluxes at a probability level of 0.05. Vertical bars in the
944 upper part represent Bonferroni and Tukey's HSD values, respectively for daily and
945 cumulative fluxes, at α = 0.05 and the presence of asterisk/s indicate significant differences
946 (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). T1: soil samples without vegetation, flooded and
947 kept under darkness. T2: soil samples with vegetation, flooded and kept under darkness. T4:

948 soil samples with vegetation, flooded and maintained in the light. T5 (control): soil samples
949 with vegetation and maintained in the light. Four replicates per treatment.
950
951 **Fig. 6** Total amount of PLFAs from soil samples (a, b), taxonomic groups (c, d) and ratios (e ,
952 f) based on PLFAs after flood stage (left) and after soil recovery stage (right) as a function of
953 flood treatments. Different letters mean differences according to Tukey's HSD test at a
954 probability level of 0.05. T1: soil samples without vegetation, flooded and kept under
955 darkness. T2: soil samples with vegetation, flooded and kept under darkness. T4: soil samples
956 with vegetation, flooded and maintained in the light. T5 (control): soil samples with
957 vegetation and maintained in the light. Four replicates per treatment.
958
959 **Fig. 7** Principal component analysis for PLFAs (taxonomic groups and ratios based on
960 PLFAs), GHG emissions, redox potential in soil, NH_4^+ and NO_3^- in soil solution after flood
961 stage (a and c) and after soil recovery stage (b and d) as a function of flood treatments. The
962 separation between treatments or biplot is shown at the left and the corresponding loading of
963 each variable included in the PCA at right. T1: soil samples without vegetation, flooded and
964 kept under darkness. T2: soil samples with vegetation, flooded and kept under darkness. T3:
965 vegetation cut from T1 before the flood stage, flooded and under kept under darkness. T4: soil
966 samples with vegetation, flooded and maintained in the light. T5 (control): soil samples with
967 vegetation and maintained in the light. Four replicates per treatment.

Table 1
Fatty acids considered in the study to establish the different taxonomic groups whose concentration is greater than 0.5% of the total PLFAs.

Taxonomic group	Fatty acid	References
Gram+ bacteria	14:0 iso, 15:0 iso, 15:0 anteiso, 15:1 iso w6c, 16:0 iso, 17:0 iso, 17:0 anteiso, 17:1 iso w9c	Ratledge and Wilkinson (1988), Kieft et al. (1994), Paul and Clark (1996), Zelles (1999), Olsson et al. (1999), Bartelt-Ryser et al. (2005)
Gram− bacteria	16:1w7c, 16:1w9c, 17:1w8c, 17:0 cyclo w7c, 18:1w5c, 18:1w7c, 18:1w9c, 19:0 cyclo w7c	Bedard and Knowles (1989), Bossio and Scow (1998), Bowman et al. (1991, 1993), Kieft et al. (1994), Paul and Clark (1996), Zelles (1999)
Actinomycetes	16:0 10 methyl, 18:0 10 methyl, 10 methyl 19:1 w7c	Zelles (1999)
Anaerobic bacteria	15:0 dma	
Protozoa	20:4w6	Paul and Clark (1996)
Fungi	18:2w6	Paul and Clark (1996)
Putative arbuscular mycorrhiza	16:1w5c	Olsson et al. (1999)
Not assigned	14:0, 15:0, 16:0, 17:0, 18:0	Ratledge and Wilkinson (1988), Niklaus et al. (2003)

Table 2
Grass above-ground dry weight and number of earthworms at the end of the soil recovery stage as a function of the flooding treatment. *P* is the ANOVA *P*-value. Different letters indicate differences between treatments according to Tukey's HSD *post hoc* test ($P < 0.05$). Four replicates per treatment.

Treatment	Grass production (g mesocosm^{-1})	Earthworms (mesocosm^{-1})
T_1: Flood + dark (no veg)	0.5 ± 0.1 c	0.0 ± 0.0 b
T_2: Flood + dark	0.6 ± 0.1 c	0.0 ± 0.0 b
T_4: Flood + light	10.9 ± 2.2 b	0.0 ± 0.0 b
T_5: Control + light	28.0 ± 1.8 a	4.8 ± 0.8 a
P	< 0.001	< 0.001

T_1: Flood + dark (no veg.): soil samples without vegetation, flooded and maintained in the dark.
T_2: Flood + dark: soil samples with vegetation, flooded and maintained in the dark.
T_4: Flood + light: soil samples with vegetation, flooded and maintained in the light.
T_5: Control + light: soil samples with vegetation, no flooding and maintained in the light.

Table 4 | Correlation matrix showing the relationship between microbially-derived phospholipid fatty acids (which constitute >2 % total of PLFAs) and soil quality parameters (greenhouse gas daily fluxes, redox potential in soil, NH_4^+ and NO_3^- in soil solution) for soils directly after flooding and after a further 5 week recovery phase.

	PLFA	15:0 iso	15:0 anteiso	16:0 iso	16:1 w7c	16:1 w5c	16:0	16:0 10 methyl	17:0 cyclo w7c	18:2 w6c	18:1 w9c	18:1 w7c	18:0	18:0 10 methyl	19:0 cyclo w7c
	After the removal of flooding														
CH_4	**−0.62**	**−0.63**	−0.44	−0.45	**−0.61**	**−0.57**	**−0.60**	**−0.69**	**−0.61**	**−0.58**	**−0.59**	**−0.58**	**−0.51**	−0.29	**−0.54**
	***P* = 0.010**	***P* = 0.009**	*P* = 0.085	*P* = 0.081	***P* = 0.013**	***P* = 0.022**	***P* = 0.015**	***P* = 0.003**	***P* = 0.012**	***P* = 0.018**	***P* = 0.016**	***P* = 0.018**	***P* = 0.043**	*P* = 0.281	***P* = 0.032**
CO_2	**−0.60**	−0.45	−0.17	−0.31	**−0.55**	**−0.50**	**−0.62**	**−0.51**	**−0.55**	**−0.77**	**−0.62**	**−0.60**	**−0.53**	−0.20	−0.35
	***P* = 0.016**	*P* = 0.083	*P* = 0.525	*P* = 0.239	***P* = 0.028**	***P* = 0.047**	***P* = 0.011**	***P* = 0.045**	***P* = 0.026**	***P* < 0.001**	***P* = 0.010**	***P* = 0.013**	***P* = 0.036**	*P* = 0.460	*P* = 0.187
N_2O	−0.08	−0.06	−0.112	−0.188	0.03	−0.03	−0.09	−0.04	−0.08	0.03	−0.14	−0.09	−0.15	−0.322	−0.20
	P = 0.763	*P* = 0.832	*P* = 0.682	*P* = 0.487	*P* = 0.907	*P* = 0.911	*P* = 0.754	*P* = 0.888	*P* = 0.776	*P* = 0.890	*P* = 0.609	*P* = 0.728	*P* = 0.572	*P* = 0.224	*P* = 0.463
Redox	**0.55**	0.32	−0.04	0.25	**0.51**	0.66	0.47	**0.69**	**0.66**	**0.82**	**0.57**	**0.56**	**0.49**	0.28	0.39
	***P* = 0.026**	*P* = 0.225	*P* = 0.877	*P* = 0.349	***P* = 0.043**	*P* = 0.006	*P* = 0.067	***P* = 0.003**	***P* = 0.005**	***P* < 0.001**	***P* = 0.022**	***P* = 0.024**	***P* = 0.053**	*P* = 0.290	*P* = 0.134
NH_4^+	**−0.78**	**−0.58**	−0.40	**−0.50**	**−0.77**	**−0.78**	**−0.77**	**−0.77**	**−0.81**	**−0.85**	**−0.74**	**−0.80**	**−0.70**	−0.32	**−0.54**
	***P* < 0.001**	***P* = 0.018**	*P* = 0.126	***P* = 0.049**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* < 0.001**	***P* = 0.002**	*P* = 0.222	***P* = 0.029**
NO_3^-	−0.22	−0.04	0.12	−0.17	−0.13	−0.17	−0.21	−0.23	−0.22	−0.34	−0.31	−0.24	−0.22	−0.27	−0.25
	P = 0.410	*P* = 0.886	*P* = 0.670	*P* = 0.54	*P* = 0.629	*P* = 0.528	*P* = 0.445	*P* = 0.392	*P* = 0.414	*P* = 0.192	*P* = 0.244	*P* = 0.372	*P* = 0.403	*P* = 0.310	*P* = 0.351
	After soil recovery														
CH_4	0.18	0.24	0.16	0.09	0.22	0.03	0.23	0.11	0.07	0.18	0.18	0.19	0.08	−0.02	0.14
	P = 0.495	*P* = 0.361	*P* = 0.551	*P* = 0.734	*P* = 0.402	*P* = 0.926	*P* = 0.383	*P* = 0.693	*P* = 0.793	*P* = 0.513	*P* = 0.513	*P* = 0.492	*P* = 0.758	*P* = 0.945	*P* = 0.600
CO_2	**0.59**	**0.54**	0.29	0.43	0.46	0.45	**0.62**	**0.56**	**0.59**	**0.64**	**0.54**	**0.59**	0.41	0.29	**0.56**
	***P* = 0.016**	***P* = 0.032**	*P* = 0.282	*P* = 0.098	*P* = 0.071	*P* = 0.078	***P* = 0.011**	***P* = 0.026**	***P* = 0.016**	***P* = 0.007**	***P* = 0.030**	***P* = 0.016**	*P* = 0.119	*P* = 0.271	***P* = 0.024**
N_2O	−0.25	0.07	−0.10	−0.15	−0.26	−0.28	−0.25	−0.05	−0.21	−0.31	−0.23	−0.25	−0.35	−0.25	−0.13
	P = 0.350	*P* = 0.808	*P* = 0.725	*P* = 0.570	*P* = 0.340	*P* = 0.293	*P* = 0.352	*P* = 0.841	*P* = 0.424	*P* = 0.236	*P* = 0.390	*P* = 0.352	*P* = 0.190	*P* = 0.346	*P* = 0.637
Redox	0.48	0.27	0.19	0.48	0.35	**0.54**	0.43	0.44	**0.55**	**0.52**	0.44	0.43	**0.54**	**0.60**	**0.60**
	P = 0.058	*P* = 0.318	*P* = 0.491	*P* = 0.063	*P* = 0.191	***P* = 0.033**	*P* = 0.098	*P* = 0.092	***P* = 0.026**	***P* = 0.038**	*P* = 0.087	*P* = 0.095	***P* = 0.031**	***P* = 0.015**	***P* = 0.013**
NH_4^+	**−0.71**	0.48	−0.41	**−0.54**	**−0.62**	**−0.64**	**−0.71**	**−0.57**	**−0.71**	**−0.73**	**−0.67**	**−0.69**	**−0.61**	**−0.46**	**−0.57**
	***P* = 0.002**	*P* = 0.064	*P* = 0.112	***P* = 0.029**	***P* = 0.011**	***P* = 0.007**	***P* = 0.002**	***P* = 0.022**	***P* = 0.002**	***P* = 0.001**	***P* = 0.001**	***P* = 0.003**	***P* = 0.011**	***P* = 0.070**	***P* = 0.020**
NO_3^-	−0.15	0.19	0.03	0.04	0.20	0.00	0.20	0.06	0.08	0.18	0.16	0.16	0.05	−0.05	0.08
	P = 0.570	*P* = 0.486	*P* = 0.917	*P* = 0.890	*P* = 0.448	*P* = 0.990	*P* = 0.456	*P* = 0.823	*P* = 0.780	*P* = 0.508	*P* = 0.554	*P* = 0.549	*P* = 0.858	*P* = 0.860	*P* = 0.762

Table 3
Correlation matrix showing the relationship between daily greenhouse gas (GHG) emissions (CO_2, N_2O and CH_4) and key soil quality parameters (redox potential, NH_4^+ and NO_3^-) during soil recovery. Significant relationships are shown in bold.

	CH_4	CO_2	N_2O	Redox	NH_4^+
CO_2	−0.013				
	$P = 0.919$				
N_2O	−0.17	0.03			
	$P = 0.181$	$P = 0.792$			
Redox	**−0.56**	0.06	0.14		
	***P* < 0.001**	$P = 0.637$	$P = 0.264$		
NH_4^+	**0.60**	0.01	0.188	**−0.58**	
	***P* < 0.001**	$P = 0.920$	$P = 0.137$	***P* < 0.001**	
NO_3^-	−0.12	−0.03	0.11	−0.05	−0.06
	$P = 0.353$	$P = 0.802$	$P = 0.396$	$P = 0.708$	$P = 0.633$

Figure 1
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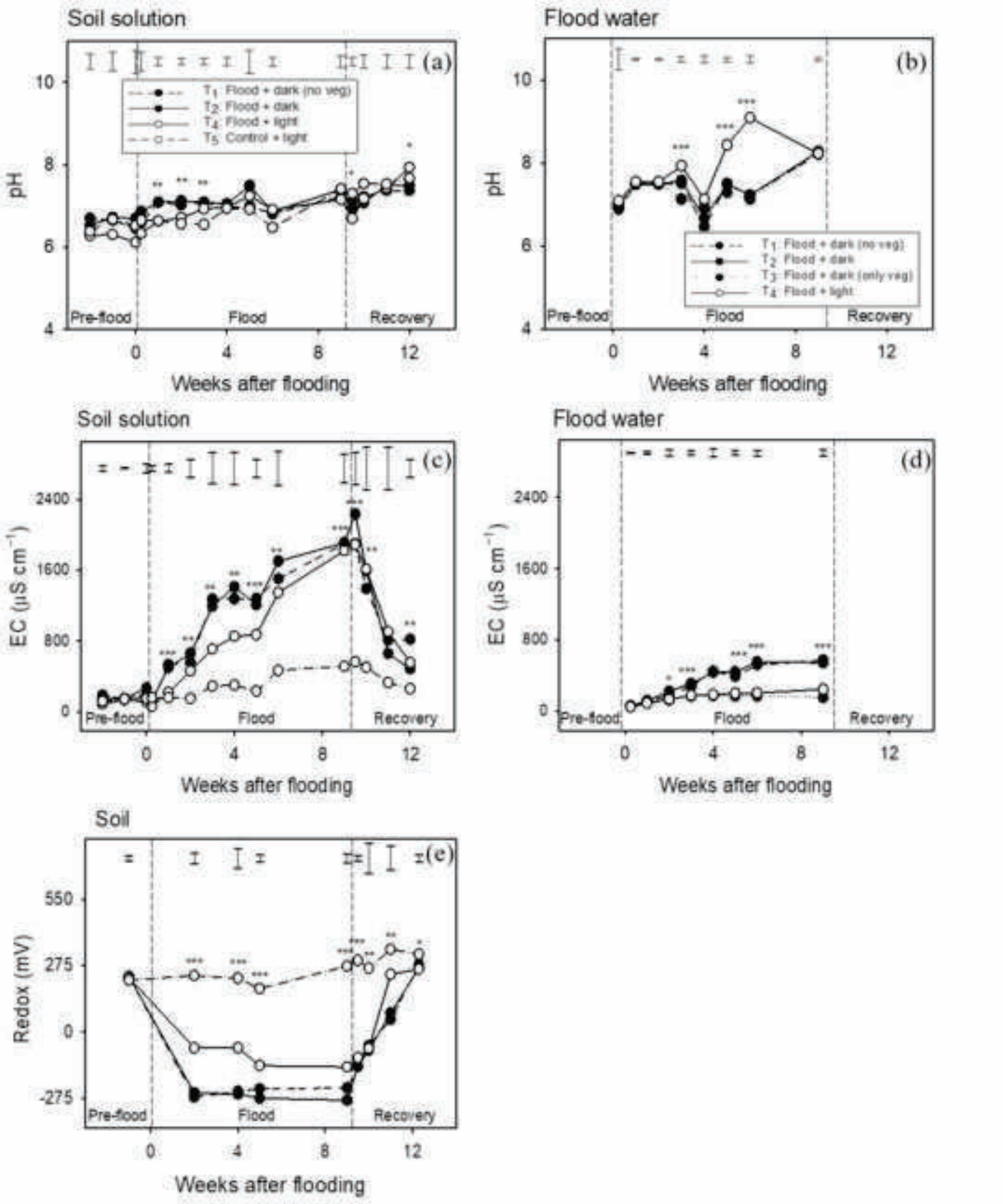

Figure 1

Figure 2
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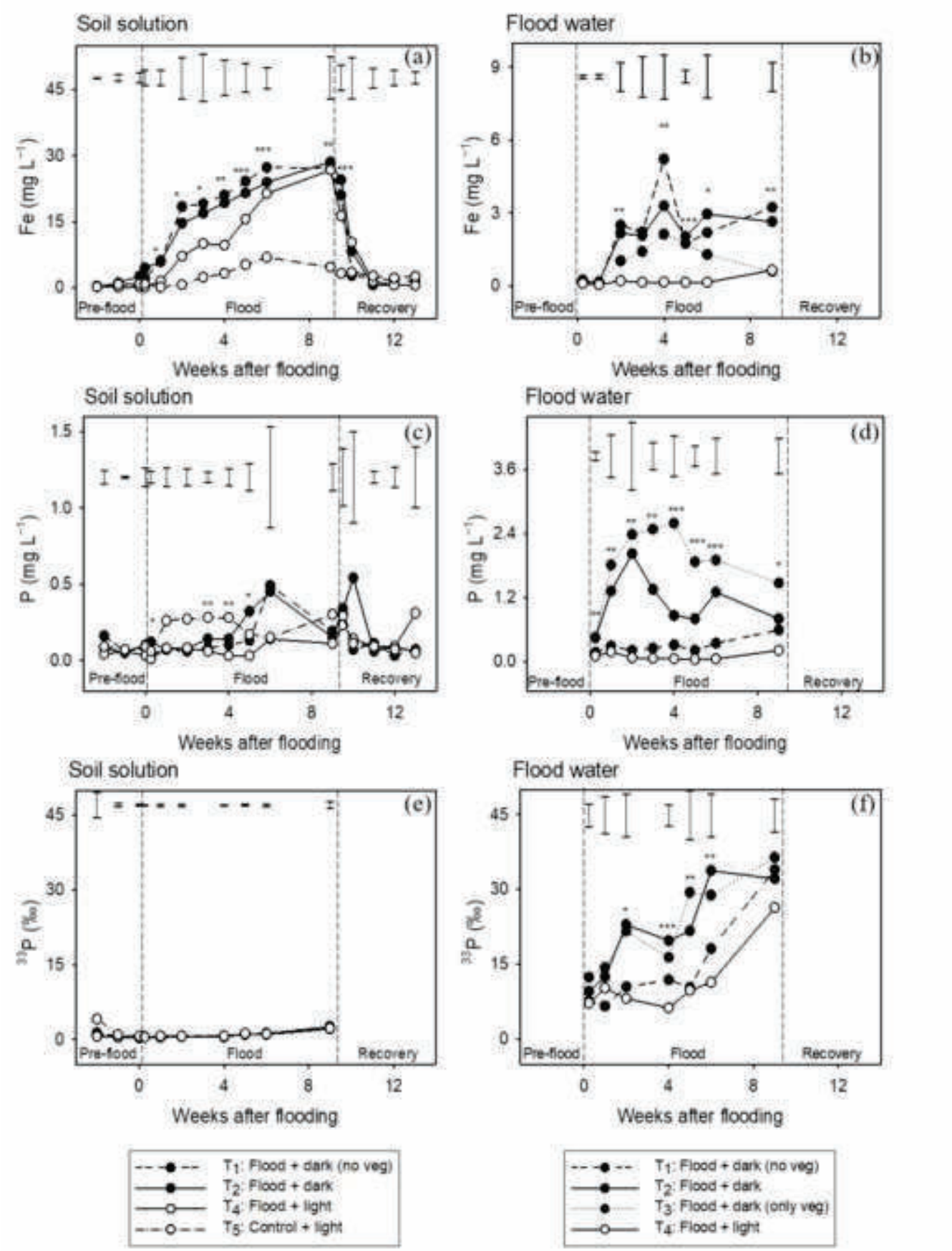

Figure 2

Figure 3
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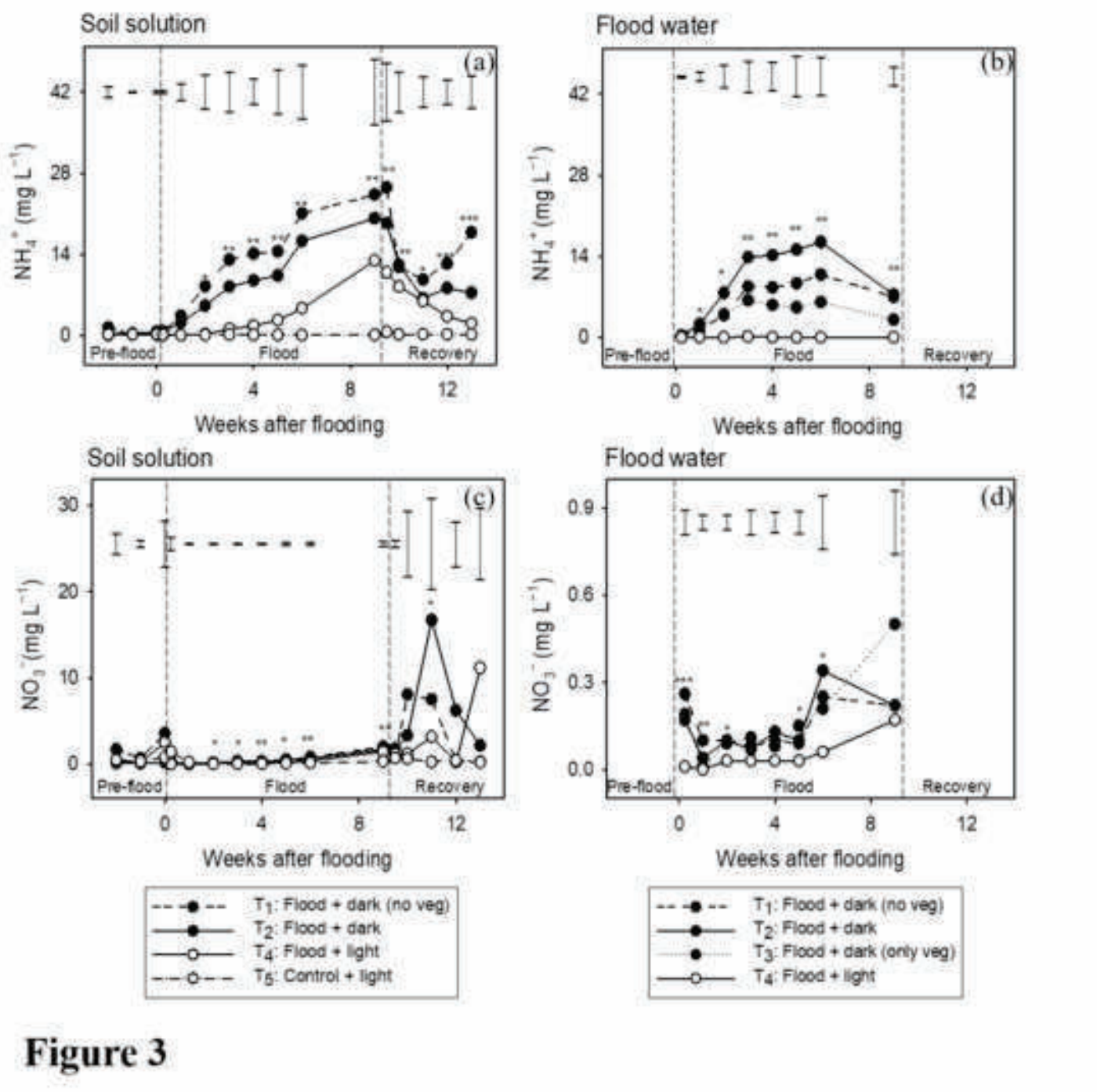

Figure 3

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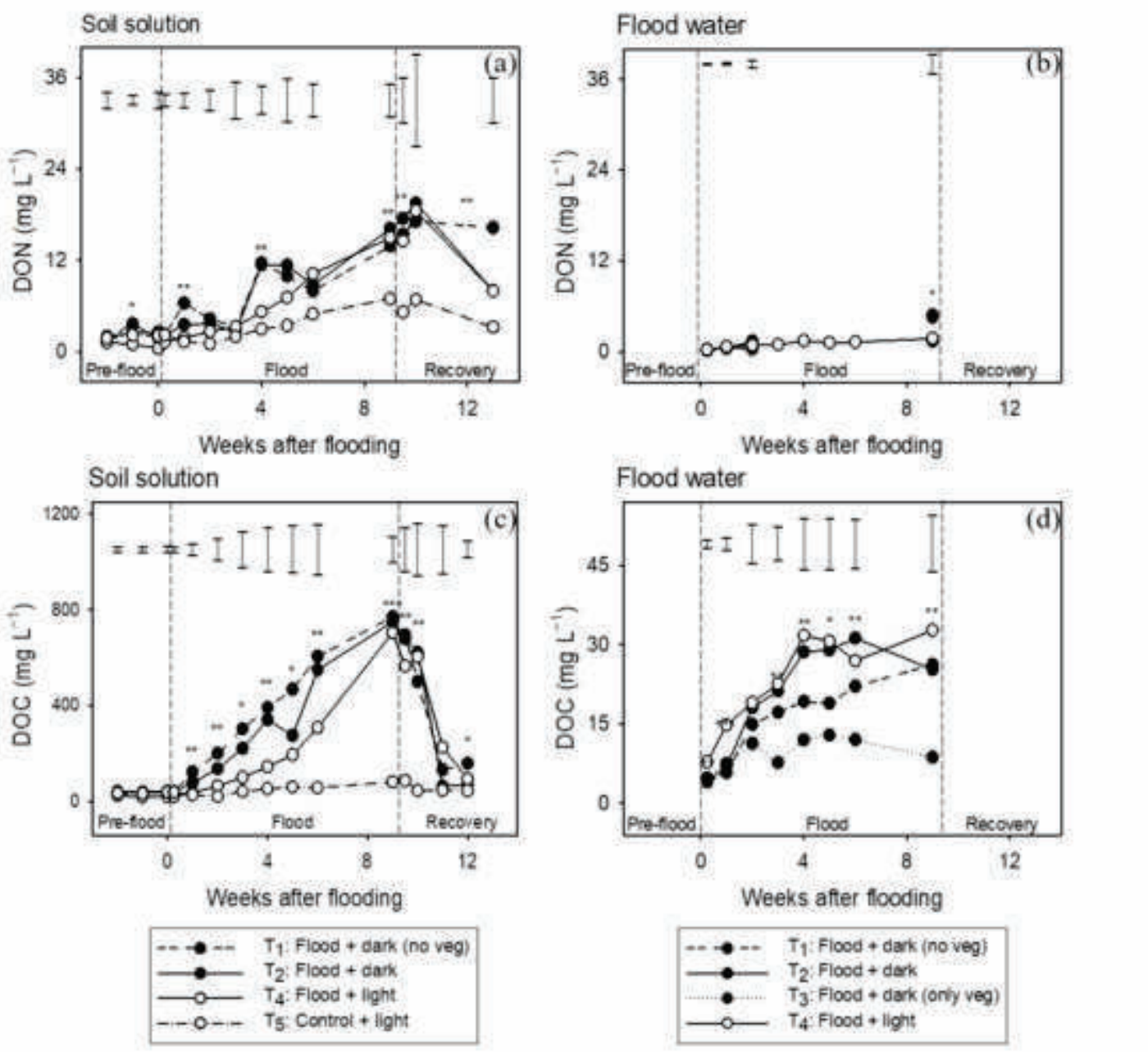

Figure 4

Figure 5
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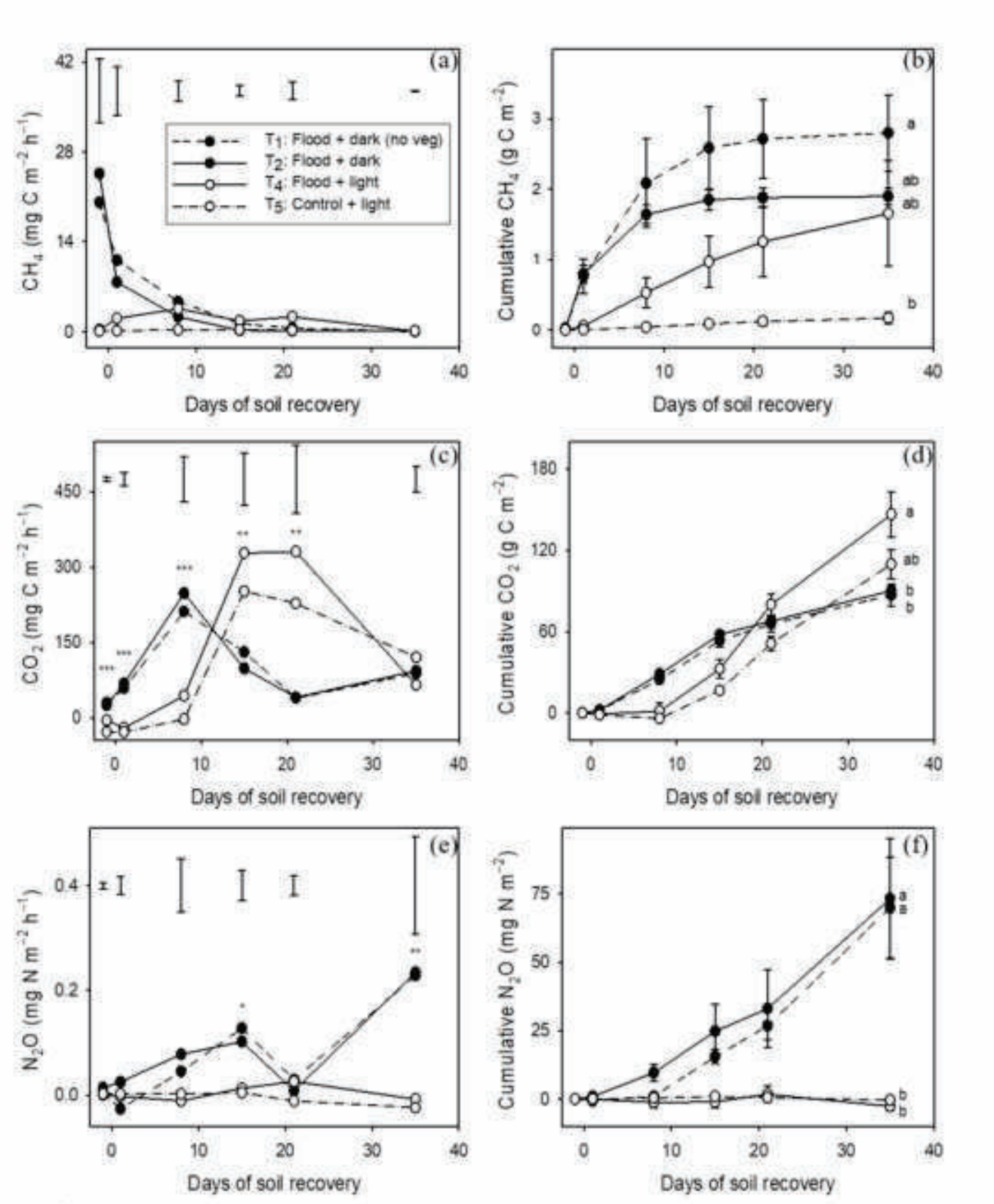

Figure 5

Figure 6

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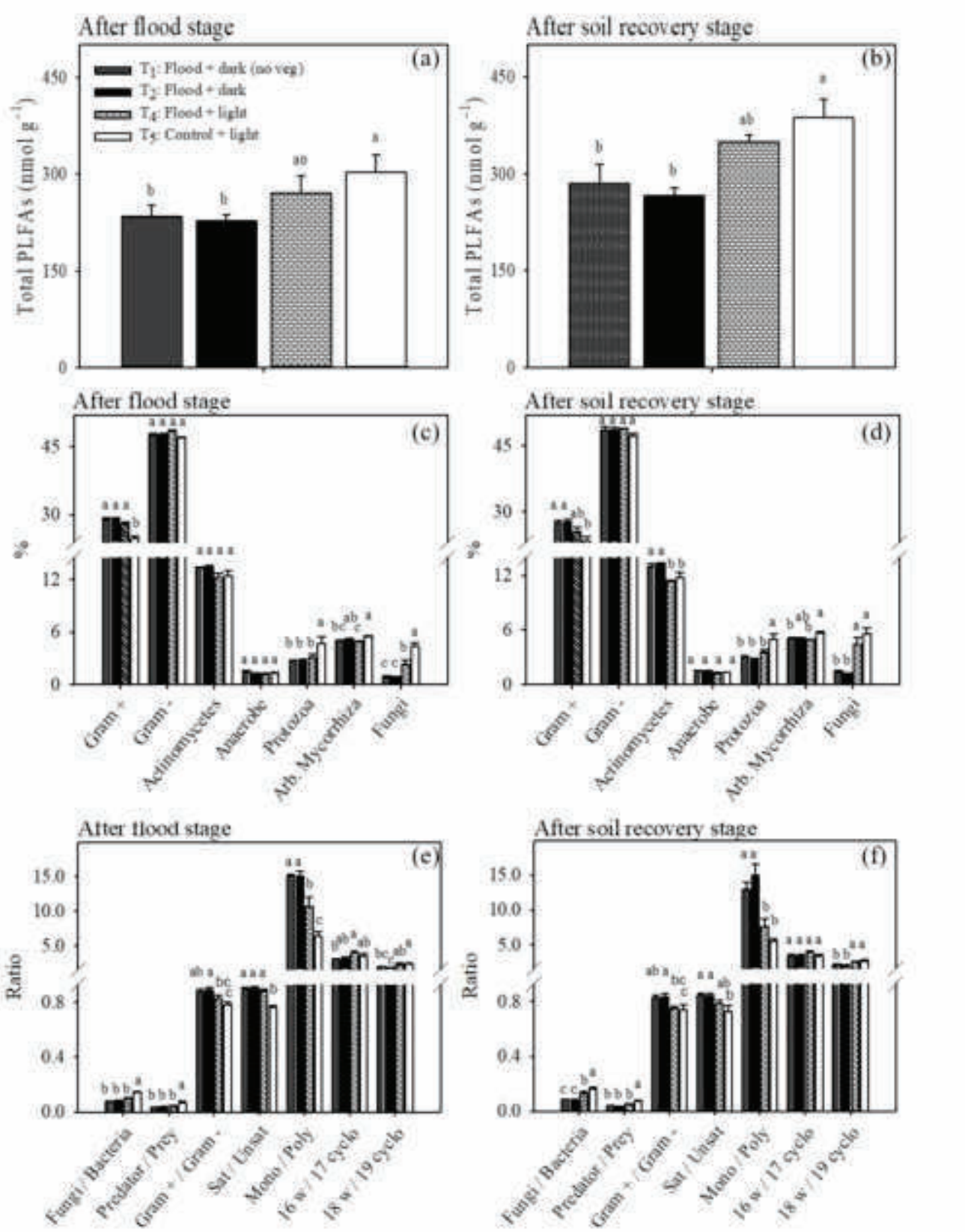

Figure 6

Figure 7
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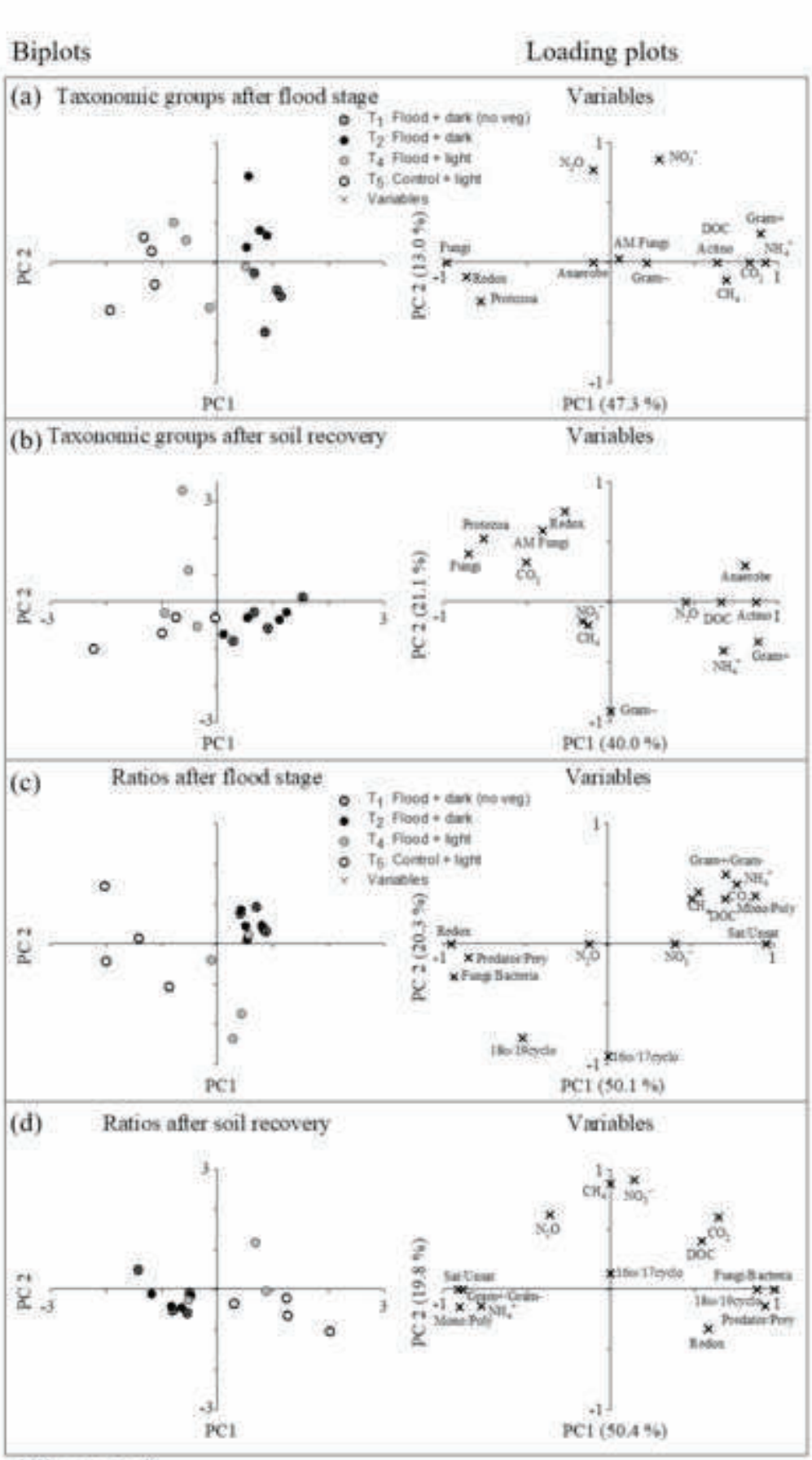

Figure 7

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